Cardiac Muscle I

1593-Pos Board B503
Transient NADH Responses to Cyanide Related to the Absorption Properties of Intact Cardiac Tissue
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Measurements of the intrinsically fluorescent metabolite NADH can be used to monitor mitochondrial redox state in isolated cardiomyocytes. NADH was 2P excited at 720nm and fluorescence was collected at 435-485nm. Calibration of the fluorescence signal with the metabolic inhibitors FCCP and cyanide enabled establishment of mitochondrial redox state. Treatment with 2μM FCCP induces maximally oxidised mitochondrial states, causing NADH fluorescence to decrease to minimal levels. Conversely, treatment with 2mM cyanide induces maximally reduced mitochondrial states, resulting in NADH fluorescence increasing to maximal levels.
Similar measurements were performed in a Langendorff perfused whole heart preparation. However, unlike with isolated cells, the cyanide response was transient in nature. A possible reason for this could be the influence of inner-filter effects in thick tissue preparations. Investigation into the effects of cyanide on tissue transmission properties was carried out using a blue LED with 450-490nm emission (corresponding to NADH emission). Cyanide perfusion caused light transmission at these wavelengths to decrease in the left ventricle (LV), but increase in the right ventricle (RV). The most likely sources of absorption at these wavelengths are from myoglobin and FAD (oxidised). Spectroscopic examination revealed that light absorption by myoglobin at wavelengths corresponding to NADH emission increased upon cyanide application (due to binding of cyanide to myoglobin). FAD absorption decreased when reduced to $FADH_2$, mimicking the effects of cyanide on mitochondrial FAD in the tissue. Thus, the additional complications caused by inner-filter effects and other optical artefacts may explain the transient NADH response to cyanide observed in our intact tissue preparations. The balance between myoglobin and FAD concentrations are likely to explain the differing transmission responses to cyanide in the LV and RV, with LV tissue containing more myoglobin.

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Drosophila as a Model for Amyloid Induced Cardiac Dysfunction
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Several human diseases are associated with the expression of mutated, misfolded and aggregation-prone amyloid proteins. Huntington's disease (HD) is caused by mutation in the Huntingtin protein that results in an expanded polyglutamine (PQ) repeat and formation of amyloid-like inclusions. The length of the PQ-repeat is also important in the progression of disease. Recent evidence indicates that patients with HD demonstrate a greater occurrence of cardiovascular events but very little is known as to how HD leads to cardiac failure. Our study uses the model organism Drosophila melanogaster to explore PQ amyloid-induced cardiac dysfunction. Using the UAS-Gal4 expression system, we express short and long UAS-PQ in the Drosophila heart using a cardiac specific driver (Hand-Gal4). Expression of extended PQ (UAS-97Q) resulted in severe cardiac defects. However, no such defects were seen upon expression of short PQ (UAS-25Q) under similar conditions. For example, the arrhythmicity index (overall measure of cardiac arrhythmia) of 1 week old PQ-97 fly hearts was twice that of the same age flies expressing PQ-25. Further, more progressive cardiac dysfunction (six fold increase in arrhythmicity index) was observed after 3 weeks in fly hearts expressing PQ-97 compared to same age fly hearts expressing PQ-25. Additionally, heart period, diastolic and systolic intervals of 1 and 3 week old fly hearts expressing PQ-97 were higher compared to same age flies expressing PQ25. Our results show that expression of PQ-97 causes progressive cardiac dysfunction. We are currently examining structural defects and possible amyloid aggregates in the cardiac tube that arises upon expression of extended PQ. Our study organism Drosophila, with its high degree of conservation relative to the human genome and many techniques to manipulate its gene expression, will be an excellent model for understanding the mechanism of cardiac failure in HD patients.

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Altered Force Development in Response to Calcium and Isopreterenol in Cardiac Muscle from Gravin Knock-Out (AKAP12) Mice
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Gravin (AKAP12), one of the A-Kinase-Binding-Proteins (AKAPs), serves as a scaffold protein linking β_2-adrenergic receptor (β_2-AR), phosphatase 2B and several kinases including Protein Kinase A (PKA) and Protein Kinase C (PKC). The presence of gravin facilitates signal transduction of β_2-AR and thus affects cardiac excitation-contraction coupling. Here, we test whether cardiac contraction is also affected in gravin knock-out (gravin-KO) mice. Trabeculae or small papillary muscles from the right ventricles were mounted between a force transducer and a motor arm, and superfused with K-H solution (pH 7.4) at room temperature. Developed force increased as external Ca^{2+} ($[Ca^{2+}]_o$) was raised from 1 to 10 mmol/L in both gravin-KO and widetype (WT) muscles. Developed force increased in a dose dependent manner as $[Ca^{2+}]_o$ was raised. In gravin-KO muscles, lower $[Ca^{2+}]_o$ (>3.0 mmol/L) caused higher but insignificant force development as compared with WT muscles. At higher $[Ca^{2+}]_o$s, developed forces remained largely flat and significantly lower in gravin-KO muscles as $[Ca^{2+}]_o$ was increased up to 10.0 mmol/L. Similarly, developed force increased as doses of isopreterenol (ISO) increased (0.05 nmol/L to 200 nmol/L) in both groups of muscles. However, force response started to blunt in gravin-KO muscle at ISO doses >5 nmol/L and became significantly lower at ISO does >50 nmol/L. These results show that gravin-KO muscles maintain their response to both Ca^{2+} and ISO, with reduced capacity at higher doses, suggesting that gravin plays an important modulatory role in the argumentation of force by Ca^{2+} and β_2-AR stimulation. Ongoing experiments are focused on changes in intracellular Ca^{2+} and myofilament Ca^{2+} responsiveness in gravin-KO mice.

1596-Pos Board B506
Gsk Mediates Rapid Estrogen-Induced Cardioprotection Without Akt Activation After Ischemia/Reperfusion
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We investigated the role of Glycogen Synthase Kinase (GSK-3β), Erk and Akt activation in rapid 17ß-estradiol (E2)-induced cardioprotection after ischemia/reperfusion.
Isolated mice hearts were retrograde-perfused using the Langendorff system at 37ºC. Hearts were perfused with oxygenated (95% O2 and 5% CO2) Krebs Henseleit solution (control) or with E2 (40 nM). After 20 min perfusion, hearts were subjected to 20 min global normothermic ischemia followed by 40 min reperfusion. Cardiac function was recorded throughout the experiment and at the end of the reperfusion (60 min) infarct size was evaluated by TTC staining. After 10 min of reperfusion, mitochondria were isolated to assess the calcium load required to induce the opening of mitochondria permeability transition pore (mPTP) referred as Calcium Retention Capacity (CRC), and whole heart lysates were prepared for Western blot analysis of pGSK-3β, pErk, pAkt and vinculin.
The E2-treated group had significantly increased CRC (287 ± 17 *vs.* 180 ± 12 nM/mg mitochondrial protein $p<0.05$), reduced infarct size ($26 \pm 3\%$ *vs.* $54 \pm 2.8\%$ $p<0.001$) and improved heart functional recovery (RPP, 11900 ± 467 *vs.* 5911 ± 318 mmHgxbeats/min, $p<0.001$) when compared to control. GSK-3β and Erk1/2 phosphorylation levels were significantly increased in E2-treated hearts (~3 and ~2 fold, respectively) without significant changes in Akt phosphorylation. These results indicate that rapid E2-induced cardioprotection inhibits the mPTP opening resulting in a reduction of the infarct size and improvement of heart function recovery via GSK-3β and Erk phosphorylation independently of Akt phosphorylation. Supported by NIH.

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Loss of Rapid Estrogen-Induced Cardioprotection in $GPER^{-/-}$ Mice After Ischemia and Reperfusion
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Several studies have demonstrated that G protein coupled receptor 30 (GPER) can directly bind to 17ß-estradiol (E2) mediating its action. Using pharmacological tools, we previously identified GPER as possible mediator of E2-mediated cardioprotection in male wild type (WT) mice subjected to ischemia/reperfusion. Here, we investigate this point further using $GPER^{-/-}$ mice.Isolated hearts from male WT (C57BL/6NCrL) or $GPER^{-/-}$ mice were perfused using the Langendorff technique with oxygenated (95% O2 and 5% CO2) Krebs Henseleit buffer (control) containing or not 40 nM E2. After 20 min of perfusion, hearts were subjected to 18 min global normothermic ischemia followed by 60 min reperfusion. Heart function was continuously recorded and myocardial necrosis was evaluated by TTC staining after the reperfusion. Mitochondria were isolated after 10 minutes of reperfusion to assess the calcium load required to induce opening of mitochondria permeability transition pore referred as Calcium Retention Capacity (CRC). In WT mice, E2 treatment induced a much better recovery of heart function when compared with untreated hearts (RPP, 11900 ± 467 *vs.* 5911 ± 318 mmHgxbeats/min, $p<0.001$). In contrast, E2 treatment had no beneficial action in GPER-/- mice. No functional differences between E2-treated and control were found (RPP, 6370 ± 655 *vs.* 6200 ± 380 mmHgxbeats/min). Similarly, the infarct size decreased significantly in WT hearts after E2-treatment ($26 \pm 3\%$ *vs.* $54 \pm 2.8\%$ $p<0.001$) but this protective infarct reduction by E2 treatment was not observed in GPER-/- hearts ($46 \pm 5\%$ *vs.* $50 \pm 4\%$). A similar pattern was found with CRC. E2 treatment in WT hearts significantly increased the